# Optimizing dopaminergic differentiation of pluripotent stem cells for the manufacture of dopaminergic neurons for transplantation.

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**Public Summary:**
BACKGROUND AIMS: We have previously described a xeno-free scalable system to generate transplantable dopaminergic neurons from human pluripotent stem cells. However, several important questions remain to be answered about our cell therapy efforts. These include determining the exact time at which cells should be transplanted and whether cells at this stage can be frozen, shipped, thawed and injected without compromising their ability to mature and survive the transplantation procedure. We also needed to determine whether further optimization of the culture process could shorten the development time and reduce variability and whether a current Good Manufacture Practice (CGMP) facility could manufacture cells with fidelity. METHODS: We developed an optimized protocol that included modulating the sonic hedgehog homolog gradient with bone morphogenetic proteins (BMP2) and addition of activin to the culture medium, which shortened the time to generate Lmx1A and FoxA2 immunoreactive cells by 4-6 days. RESULTS: We showed that cells at this stage could be safely frozen and thawed while retaining an excellent ability to continue to mature in vitro and survive transplant in vivo. Importantly, we successfully adapted this process to a CGMP facility and manufactured two lots of transplant-ready dopaminergic neurons (>250 vials) under CGMP-compatible conditions. In vitro characterization, including viability/recovery on thawing, whole genome expression as well as expression of midbrain/dopaminergic markers, showed that the cells manufactured under GMP-compatible conditions were similar to cells produced at lab scale. CONCLUSIONS: Our results suggest that this optimized protocol can be used to generate dopaminergic neurons for Investigational New Drug enabling studies.

**Scientific Abstract:**
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